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further consideration by the Examiner. The claims under consideration currently are Claims 1 to 5 and 30. No claims have been cancelled, added, or amended by the present Reply. No new matter has been added.

Discussion of the Examiner's Obviousness Rejection of Claims 1 to 5 and 30

In the present Action, the Examiner rejected each of the elected pending claims (Claims 1 to 5 and 30) under 35 U.S.C. §103 as being unpatentable in view of the combined disclosures of Bruno et al., *Cancer Surveys*, 17: 305-313 (1993), (hereafter "Bruno et al.") and Urien et al., *Invest. New Drugs*, 14: 147-151 (1996), (hereafter "Urien et al."). These rejections were repeated from the February 11, 2003 Action and made final.

Applicant, however, believes that the arguments presented in the previous June 11, 2003, Reply remain valid and submit respectfully that the Examiner has misunderstood applicant's arguments. Accordingly, these arguments are re-presented here along with documents which further support these arguments. Applicant requests respectfully that these arguments be fully considered.

Applicant submits that, contrary to the Examiner's assertion in his Action, applicant has indeed considered the Bruno et al. and Urien et al. references in combination when traversing the Examiner's rejection. In particular, applicant fully considered the combined disclosures of these two references and concluded that the combined disclosures of such references fail to render applicant's claims obvious since the Examiner failed to establish a *prima facie* case of obviousness.

To establish a *prima facie* case of obviousness, the following criteria must be

met: (A) the combined references must teach or suggest all the claim limitations; and (B) there must be a reasonable expectation of success. MPEP §2143. As discussed below, neither of these two criteria have been met.

In the previous Reply, applicant argued that one skilled in the art, even if he/she could have arrived at applicant's method from the combined disclosures of Bruno et al. and Urien et al., would not have had a reasonable expectation that such a method would be successful. This is because, while AAG may be the main determinant of docetaxel plasma binding variability and docetaxel clearance, these properties alone do not render obvious measuring AAG levels in a patient to determine what dose of a taxoid to administer to a patient. The Examiner's attention is directed to the enclosed pages 20 and 21 of Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (1990 Pergamon Press, Inc.) which states that the important factors in determining what dosage of a drug to administer to a patient are: (A) clearance; (B) volume of distribution; and (C) bioavailability. Each of these factors are unpredictable and can play a role in determining dosage of a given drug. While the combined disclosures of the cited references may disclose that AAG is the main determinant of docetaxel clearance and serum binding variability, the combined disclosures do not address the importance of the other two major factors in determining the proper dose of taxoid to administer to a patient nor do they indicate that these factors may be ignored in determining dosage of a taxoid. Without a discussion as to the effects on volume distribution and bioavailability, one skilled in the art would not derive from the combined disclosures of the cited references that AAG would be the basis to determine the proper dose of taxoid to administer to a patient. Accordingly, one skilled in the art would not have had any reasonable expectation that applicant's method for determining the amount of taxoid to administer to a patient based on that patient's AAG level would be successful.

Applicant notes further that, even if the Examiner persists in the belief that clearance is the sole factor relevant in the determination of a proper dose of taxoid, the state of the art at the time of applicant's invention was that it had not yet been settled as to whether AAG really was the main determinant of docetaxel clearance, as claimed by Urien et al. Applicant would like to direct the Examiner's attention to Marre et al., *Cancer Research*, 56: 1296-1302 (1996), (copy enclosed), which was published in the same year as Urien et al. Marre et al. states that CYP3A is a major influence on docetaxel clearance in humans. Also, Hirth et al., *Clinical Cancer Research*, 6: 1255-1258 (2000), (copy enclosed), published after Urien et al. and after the filing date of the present application, states that, even in 2000, it was still unclear whether CYP3A or AAG was the main determinant of docetaxel clearance. Thus there was a significant level of uncertainty as to whether AAG really is the major determinant of docetaxel clearance. In addition, it should be noted that the combined disclosures of Bruno et al. and Urien et al. do not disclose whether AAG is a determinant of the clearance of any taxoid other than docetaxel. Marre et al. states that CYP2C8/9, not CYP3A, is a major determinant of the clearance of paclitaxel (another taxoid). Accordingly, even if the Examiner were to take the position that clearance is the sole factor relevant in the determination of a proper dose of taxoid, it was still unclear as of 2000 what protein is the main determinant of docetaxel clearance. Further, one skilled in the art would have known that, even if AAG were the main determinant of docetaxel clearance, it would not necessarily be the main determinant of the clearance of taxoids other than docetaxel.

Given the uncertainty in the state of the art at the time of applicant's invention, even if one skilled in the art takes the Examiner's position that clearance is the sole factor relevant in the determination of a proper dose of taxoid, he/she still would not have had any reasonable expectation that applicant's method for determining the amount of taxoid to administer to a patient based on that patient's AAG level would be

successful.

In view of the above, it is clear that the combined disclosures of the cited references are inconclusive with respect to determining the proper dose of a taxoid to administer to a patient. Accordingly, one skilled in the art, upon reviewing the combined disclosures of Bruno et al. and Urien et al., would still have no reasonable expectation of success that applicant's method would be successful in determining a proper dosage of taxoid for administration to a patient.

Independent of the above argument, applicant, in the previous Reply, presented a further argument as to why the Examiner has failed to establish a *prima facie* case of obviousness. Specifically, the combined disclosures of Bruno et al. and Urien et al. do not teach or suggest all of the claim limitations of the rejected claims. Claims 1 to 5 and 30 are directed to a method for determining the dosage of a taxoid to administer to a patient being treated for cancer. Various steps for such a method are recited in the claims, namely: (A) observing the patient's level of AAG; (B) evaluating said level to determine the dosage of taxoid to administer to said patient by comparing said level to a predetermined AAG level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said evaluation, recommending the dosage of the taxoid to administer to the patient. The combined disclosures of the cited references do not teach or suggest these steps. The Examiner, in his Action, has not addressed this aspect of applicant's Reply and applicant believes this argument to remain valid as well.

Given the above, applicant requests respectfully that the Examiner withdraw his rejection of Claims 1 to 5 and 30. If the Examiner persists in his rejection, applicant requests, in order to clarify the record in the event of an Appeal, that the Examiner present reasons as to why applicant's arguments fail to traverse successfully the

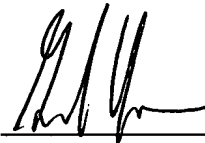
Examiner's rejection.

Conclusion

For the reasons expressed above, applicant requests respectfully that the Examiner reconsider and withdraw his rejections under 35 U.S.C. §103.

In view of the foregoing remarks, an early and favorable action is requested respectfully.

Respectfully submitted,



Gene J. Yao, Esquire
Reg. No. 47,193
Attorney for Applicant

Synnestvedt & Lechner LLP
Suite 2600 Aramark Tower
1101 Market Street
Philadelphia, PA 19107-2950
(215) 923-4466

The Effect of an Individual's Cytochrome CYP3A4 Activity on Docetaxel Clearance¹

JoAnn Hirth, Paul B. Watkins,
Myla Strawderman, Anne Schott, René Bruno,
and Laurence H. Baker²

Division of Hematology/Oncology [J. H., A. S., L. H. B.], General Clinical Research Center [P. B. W.], and Biostatistics Core [M. S.], University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan 48109, and Rhône-Poulenc Rorer, Antony Cedex 92165, France [R. B.]

ABSTRACT

Docetaxel is a chemotherapeutic agent effective in the treatment of various solid tumors. Patients given a standard dose of docetaxel exhibit wide interpatient variation in clearance (CL) and toxic effects. Docetaxel undergoes metabolism by cytochrome CYP3A4. Thus, interpatient variability in CYP3A4 activity may account in part for differences in toxicity and CL. Twenty-one heavily pretreated patients with metastatic sarcomas received docetaxel (100 mg/m²). Hepatic CYP3A4 activity in each patient was measured by the [¹⁴C-N-methyl]erythromycin breath test (ERMBT). Blood samples were taken at selected times over the next 24 h for pharmacokinetic analysis. Phenotypic expression of hepatic CYP3A4 activity measured by the ERMBT varied over 20-fold (administered ¹⁴C exhaled in 1 h: mean, 2.53%; range, 0.25–5.35%), which is similar to a normal control population. CL of docetaxel varied nearly 6-fold (mean, 21.0 liters/h/m²; range, 5.4–29.1 liters/h/m²). The ERMBT was the best predictor of CL when compared with serum alanine aminotransferase, albumin, alkaline phosphatase, or serum α -1-acidic glycoprotein. The natural log of ERMBT accounted for 67% of the interpatient variation in CL. Multivariate analysis showed that the natural log of ERMBT and albumin together accounted for 72% of the interpatient variation in CL. The greatest toxicity was seen in patients with the lowest ERMBT. Hepatic CYP3A4 activity is the strongest predictor of docetaxel CL and accounts for the majority of interpatient differences in CL. Patients with low CYP3A4 activity are at risk for having decreased CL and may thus experience increased toxicity from docetaxel. Those with high activity may be receiving a suboptimal dose. By measuring CYP3A4 activity, the ERMBT may be clinically

useful in tailoring doses of CYP3A4 substrates, such as docetaxel, in certain individuals.

INTRODUCTION

Patients and healthy individuals alike display significant differences in terms of efficacy and adverse effects after exposure to many drugs. An identical dosage of a drug can result in widely different concentrations of the therapeutically active compound or metabolites (1). The presence of genetic variability in the activity of enzymes involved in drug metabolism could lead to clinically important variations in efficacy or adverse effects for some drugs.

The cytochrome P450 system is a family of heme-containing enzymes responsible for the metabolism of nonendogenous substances, such as drug molecules and toxins (2). Specific isoenzymes are designated by the prefix "CYP," and to date, over 30 isoenzymes have been purified, cloned, sequenced, and characterized in humans. CYP3A4 is found in the liver and small bowel. The CYP3A4 enzyme displays large interpatient differences in both content and catalytic activity in humans (3). These differences exist even in the absence of medications known to induce or inhibit the enzyme and are thus likely to reflect genetic variability (3).

The ERMBT³ specifically measures the *in vivo* activity of hepatic CYP3A4 (3, 4) and is therefore ideally suited for drugs given *i.v.*, such as docetaxel. This test is based on the fact that CYP3A4 is the major enzyme responsible for erythromycin metabolism, liberating a carbon atom that is exhaled as carbon dioxide. After injecting a trace dose of radiolabeled erythromycin, the rate of radiolabeled carbon dioxide exhaled is measured. This test has been validated as a specific measure of CYP3A4 activity (3).

In vitro studies suggest that CYP3A4 is the major enzyme involved in docetaxel metabolism (5). Docetaxel is excreted in the feces with less than 8% excreted unchanged (6). It is highly bound to plasma proteins including AAG. Pharmacokinetic data show significant variation in CL between patients (7). Decrease in total body CL is associated with increased frequency and severity of side effects (8). Multivariate analysis demonstrates that interpatient variability of CL is predicted by body surface area, AAG plasma level, and elevated hepatic enzymes; however, these parameters do not account for total variability (7). Because docetaxel binds chiefly to AAG, high levels of this protein presumably limit the free fraction of docetaxel available for CL in the liver. Due to lower CL of docetaxel in patients with elevated transaminases, it is currently recommended in the United States package insert that patients with an ALT > 1.5×

Received 8/24/99; revised 12/1/99; accepted 12/6/99.

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¹ Supported by General Clinical Research Center Grant M01 RR00042.

² To whom requests for reprints should be addressed, at University of Michigan Comprehensive Cancer Center, 1500 East Medical Center Drive, 7216 CCGC, Ann Arbor, MI 48109-0948.

³ The abbreviations used are: ERMBT, erythromycin breath test; AAG, α -1-acidic glycoprotein; ULN, upper limit of normal; ALT, alanine aminotransferase; ln ERMBT, natural log of ERMBT; GCRC, General Clinical Research Center; CL, clearance.

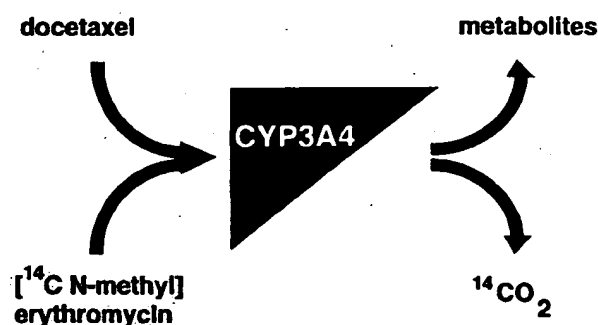


Fig. 1 Drug metabolism via cytochrome CYP3A4.

the ULN concomitant with alkaline phosphatase $> 2.5 \times$ ULN or bilirubin $> \text{ULN}$ do not receive this drug. The European Summary of Product Characteristics alternatively recommends a dosage reduction of 25% in patients with concomitant elevations of ALT and alkaline phosphatase.

Previous studies have shown modest activity for docetaxel in patients with advanced sarcomas (response rates of 17% and 18%), thus we decided to try this agent in our sarcoma population (9, 10). We hypothesized that variability in CYP3A4 activity accounts for interpatient differences in docetaxel CL and toxicity. Thus, the ERMBT was used to measure CYP3A4 activity in sarcoma patients receiving docetaxel (Fig. 1).

PATIENTS AND METHODS

Study Population. Twenty-one patients with measurable, metastatic sarcomas of bone or soft tissue origin were entered into our trial. Eighteen patients had soft tissue sarcomas, whereas three had bone sarcomas. Twelve patients were men, and nine were women (age range, 19–72 years). Thirteen of the patients with soft tissue sarcomas had received extensive prior chemotherapy, whereas five patients with gastrointestinal stromal sarcomas had not received extensive prior chemotherapy. Two of the three patients with bone sarcomas had also received extensive prior chemotherapy. All patients gave written informed consent, and the study was approved by the Protocol Review Committee of the Cancer Center and the Review Committee of the GCRC, as well as the University of Michigan Institutional Review Board. All patients had normal hematological values and liver chemistries within the limits recommended for the use of docetaxel at the time of admission (normal total bilirubin, alkaline phosphatase $< 5 \times$ the ULN, and serum transaminase levels $< 1.5 \times$ the ULN). Concurrent use of drugs known to alter CYP3A4 activity such as imidazole antimycotic agents (fluconazole), macrolide antibiotics (erythromycin), antiseizure medications (phenobarbital and phenytoin), and rifampin were specifically avoided. Other exclusion criteria were concurrent radiotherapy, active infection, pregnancy or lactation, and allergy to erythromycin.

Study Schema and Procedures. Three ERMBTs were administered to each patient at the GCRC. The test was done on three occasions in case either the dexamethasone premedication or the chemotherapy introduced variability. The first study served as a baseline and was done before admission for docetaxel (range, 1–19 days before admission). The second study was done 24 h after

dexamethasone premedication was initiated and 1 h before the infusion of docetaxel. The third study was done 3 h after the infusion of docetaxel had been completed. A total of 3 μCi (< 0.1 mmol) of [¹⁴C-N-methyl]erythromycin obtained from Metabolic Solutions, Inc. (Nashua, NH) was given i.v. Exhaled carbon dioxide was trapped in a solution of hyamine hydroxide, ethanol, and a blue indicator before and 20 min after the injection. The percentage of ¹⁴C exhaled/h = $49.496 \times$ (percentage of ¹⁴C exhaled/min at 20 min – baseline counts) (11).

Docetaxel was administered at 100 mg/m² i.v. over 1 h. Each patient received the following standard premedications: (a) dexamethasone (8 mg, p.o.) every 12 h starting the day before infusion and continuing for 5 days; and (b) granisetron (2 mg, p.o.) 30 min before the infusion. Blood was drawn for pharmacokinetic analysis over 24 h with specimens at optimal sampling times: just before the end of infusion and at 0.25, 0.75, 3.00, 6.50, and 24.00 h after the end of infusion (12). Case report forms documented actual sampling times as well as the actual beginning and end times of the infusion. Docetaxel was assayed from frozen plasma samples using reverse-phase high-performance liquid chromatography (13). All samples were assayed at the same time. A Bayesian criterion was used to calculate the docetaxel plasma concentration area under the curve based on measured drug levels and population pharmacokinetic parameters using NONMEM software (12, 14). The area under the curve was computed as dose/CL. CL was directly estimated by fitting the model (12, 14). Patients had baseline liver chemistries and serum AAG levels drawn. One patient did not have a baseline serum ALT recorded, and the two patients had insufficient sample to determine the AAG level. Baseline and weekly hematological counts were checked. Granulocyte-colony stimulating factor was not given during this first cycle.

Subsequent cycles of chemotherapy were given every 3 weeks if the patient was judged by us to be deriving clinical benefit. The pharmacokinetic data were obtained only during the initial cycle. Treatment was stopped if the disease progressed, if intolerable toxicities developed, or at the patient's request. Radiological evaluation of patients was done at the discretion of the treating physician to document clinical response or progression.

Statistical Analysis. Although the distribution of the ERMBT in a large sample of normal controls is positively skewed, the distribution in this small sample is approximately normal. The paired *t* test was used to compare the breath test values obtained at the three time points. Pearson's correlation coefficient (*r*) further describes the strength of the linear relationship and is presented along with the *P*. Nonparametric analogues to these test procedures were conducted for confirmation but are not reported.

CL, as determined by pharmacokinetic modeling, is approximately normal. The association between CL and patient characteristics was evaluated using simple and multiple least-squares regression models. Variables considered as potential predictors of CL in this model included ERMBT, AAG, albumin, ALT, and alkaline phosphatase. A natural log transformation was applied if indicated by univariate residual plots. For instance, the relationship between the ERMBT and CL was not linear over the entire range of values, particularly at the lowest ERMBT values, and the low ERMBT values had a better fit when \ln ERMBT was used. The best model for predicting CL was determined from the set of predictors using a step-wise procedure based on the largest *F*

Table 1 Variability in breath tests and docetaxel clearance

	Mean	SD	Range
ERMBT #1 ^a	2.41	1.08	0.125–4.86
ERMBT #2	2.53	1.17	0.41–5.35
ERMBT #3	2.25 ^b	0.93	0.53–4.37
Docetaxel CL ^c	20.42	6.30	5.4–29.1

^a ERMBT = percentage of ¹⁴C exhaled/h.^b Significantly lower than ERMBT #2 ($P = 0.0047$).^c Docetaxel CL is given in liters/h/m².

statistic for each variable's contribution to the model. The first factor included using the step-wise procedure is the best single predictor of CL. Two patients missing certain lab test results were excluded from analyses that involved these lab tests. All two-way interactions were examined for the final best model. All analyses were conducted using the SAS software package.

RESULTS

The interpatient variability in ERMBTs and docetaxel CL is evident in the range of values obtained, as shown in Table 1. The ERMBT varied >20-fold, largely because a few patients had very low breath test results. The CL of docetaxel varied >5-fold among these patients. These ERMBT data exhibit similar variability to that seen in 107 normal controls studied previously (ERMBT mean = 2.56; SD, 1.06; range, 0.77–6.15). There was excellent correlation between ERMBT results on all three occasions ($r = 0.81$ – 0.92 , $P < 0.0001$). Thus, there is no evidence for a significant effect of dexamethasone premedication on the ERMBT. There is a tendency for the test value to fall modestly after docetaxel administration (ERMBT #3 versus ERMBT #2, $P = 0.0047$), likely reflecting competition between erythromycin and docetaxel for metabolism by CYP3A4. The ERMBT #2 was used in subsequent analyses.

Fig. 2 demonstrates the relationship between CL and the phenotypic measurement of CYP3A4. As expected, those patients with reduced CL had the lowest ERMBT results. As mentioned in the statistical section, the low ERMBT values had a better fit when \ln ERMBT was used, with $r^2 = 0.67$, $P = 0.0001$. Even if the ERMBT was not log-transformed, the relationship between CL and the ERMBT remained strong ($r^2 = 0.49$, $P = 0.001$). Albumin was also a good predictor of CL ($r^2 = 0.46$, $P = 0.001$). Alkaline phosphatase was not as strong a predictor of CL. One patient with osteosarcoma had an exceedingly high alkaline phosphatase (5769) from bone rather than liver origin and was therefore included in the study. When this outlier was removed from the analysis, alkaline phosphatase was a weak predictor of CL ($r^2 = 0.36$). The relationships between CL and ALT or AAG were also weak ($r^2 = 0.23$ and 0.12 , respectively), but in the anticipated inverted direction. Thus, the ERMBT proved to be the best single predictor of docetaxel CL.

Virtually all patients had National Cancer Institute grade 3 or 4 neutropenia at the nadir (83% grade 4; 6% grade 3). Blood counts were only checked weekly, so it is possible that the nadir was missed on a couple of patients. It is also interesting to note that the two patients requiring hospitalization with the severest toxicities (mucositis/sepsis/neutropenia) were those with the lowest ERMBT and docetaxel CL results (5.4 and 10.5 liters/h/m²). The AAG levels, which were shown previously to be a

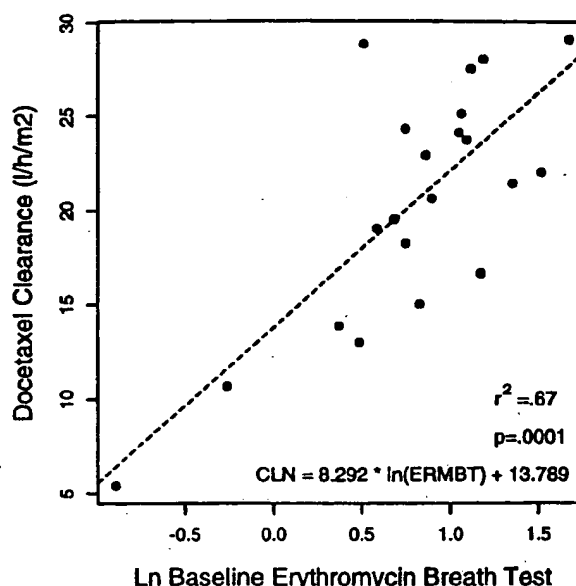


Fig. 2 Relationship between CL and phenotypic measurement of CYP3A4.

good predictor of CL and toxicity (8), showed no trend in predicting this toxicity, and both patients were within the recommended range for liver chemistries.

As described in the statistical section, simple and multiple regression analyses were conducted to model the best predictors of CL. The best two-factor model for predicting CL was determined to be \ln ERMBT and albumin ($r^2 = 0.72$). No other factors added significantly to this model, and no statistically significant codependence between variables was detected. In the two-factor model containing untransformed ERMBT and AAG, both variables were independently predictive of CL ($r^2 = 0.61$), with higher AAG predicting lower CL.

DISCUSSION

Both the CL of docetaxel and CYP3A4 activity, as measured by the ERMBT, had marked interpatient variation in this study. All patients were required to have good hepatic function as described previously, and with the exception of dexamethasone, no patient received known potent inhibitors or inducers of CYP3A4. Thus, it appears that the variation seen could reflect genetic differences in the metabolism of docetaxel.

The most significant independent variable examined to predict docetaxel CL in this study was hepatic CYP3A4 activity as measured by ERMBT. Previously, the best predictors of docetaxel pharmacokinetics that had been identified were abnormal liver chemistries and serum AAG level. Albumin was also a significant predictor of CL, but its effect was of low magnitude compared with that of AAG (9). In this study, AAG and the ERMBT results do not appear to be correlated, and the ERMBT is a superior predictor of CL. In fact, the ERMBT and albumin are the strongest predictors of CL, and together they account for 72% of the interpatient variability in CL. Why is the AAG a poorer predictor in this study? The pharmacokinetic data and AAG levels in this study were deter-

mined in the same manner and by the same investigator as these prior data, so quality control should not be a factor. The small sample size of the present study may account for this difference. Bruno's study (8) included 547 patients and used a statistical approach with Bayesian estimates directed toward subpopulations with extreme covariate values (presumably clinically at a higher risk). This requires a large sample size. This approach is not applicable to our study of 21 patients. The variability of AAG levels may also be important. Docetaxel is strongly protein-bound to AAG, thus high AAG levels should decrease docetaxel CL. In the previous work (7), this was evident for high AAG levels; however, the model did not improve the prediction of CL for patients with low AAG levels (<0.88 g/liter). Perhaps our study did not include as many patients with "high" AAG levels, and thus AAG was not found to be an important predictor of docetaxel CL. The AAG reflects the free fraction of drug available for CL in the liver, whereas CYP3A4 activity reflects the individual's inherent ability to metabolize the drug. Although this is a small study, the fact that CYP3A4 activity was a better predictor is an important new finding and will hopefully improve our understanding of the pharmacokinetics of docetaxel. Larger studies are under way and will help clarify the importance of AAG and CYP3A4 activity in relationship to CL.

Although toxicities (namely, grade 3 or 4 neutropenia) were seen in virtually all patients at this dose level, the observation that an ERMBT result of <1 in this study was associated with the severest toxicities is interesting. Although marked interpatient heterogeneity in the expression of CYP3A4 genes is known to exist, the clinical implications of having a "high" or "low" CYP3A4 activity were not necessarily known. In this study "low" CYP3A4 activity (ERMBT < 1) appears to be associated with excess toxicity. It implies that consideration should be given to modifying the dose for patients with such a low ERMBT. Similarly, one would wonder whether patients with "high" CYP3A4 activity are at risk of getting subtherapeutic doses of CYP3A4 substrates such as docetaxel. This hypothesis should be studied further.

In this study, single agent docetaxel was given, but docetaxel is now commonly included in combination regimens. Because the additional drugs are often myelotoxic but not CYP3A4-mediated, the ERMBT may be helpful in sorting out the specific effect docetaxel makes with regard to the toxicity of the regimen.

Logistically, the ERMBT is easy to administer and poses a minimal risk to patients. It proved to be highly reproducible. It was not significantly affected by dexamethasone premedication, and this observation is consistent with prior data (15) that showed no substantial effect of dexamethasone premedication on docetaxel CL. The test can therefore be done at a convenient time, days or weeks ahead of the planned administration of docetaxel.

In conclusion, CYP3A4 activity was found to be the most significant independent variable for predicting the CL of docetaxel. The ERMBT, perhaps together with serum albumin or AAG, may be clinically useful in individualizing the dose of docetaxel and possibly other drugs that are CYP3A4 substrates, including several new antineoplastic agents. It may also be helpful in individualizing the dose of docetaxel in patients with markedly elevated hepatic enzymes who are currently excluded from treatment with docetaxel.

We plan to initiate a study using tailored dosing of docetaxel based on CYP3A4 activity. We postulate that by tailor-

ing the dose, variability in patient drug exposure could be diminished. Hopefully, modern pharmacogenetics can be put into practical use in the clinic and allow us to treat patients with better precision and less toxicity.

ACKNOWLEDGMENTS

We thank Mary Vestich for secretarial support, Michelle Walsh for technical assistance, and Marlene Woo for clinical assistance. We are also very grateful for the contributions of A. Joly and P. Baille (Rhône-Poulenc Rorer, Antony, France) with regard to the pharmacokinetic analysis of docetaxel. Finally, we thank the GCRC staff, without whom this study could not have been done.

REFERENCES

1. Linder, M. W., Prough, R. A., and Valdes, R., Jr. Pharmacogenetics: a laboratory tool for optimizing therapeutic efficiency. *Clin. Chem.*, **43**: 254-266, 1997.
2. Kivistö, K. T., Kroemer, H. K., and Eichelbaum, M. The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interaction. *Br. J. Clin. Pharmacol.*, **40**: 523-530, 1995.
3. Watkins, P. B. Noninvasive tests of CYP3A enzymes. *Pharmacogenetics*, **4**: 171-184, 1994.
4. Turgeon, D. K., Leichtman, A. B., Blake, D. S., Schmouder, R. L., Lown, K. S., Annesley, T. M., and Watkins, P. B. Prediction of interpatient variation in OG-37-325 dosing requirements by the erythromycin breath test. *Transplantation (Baltimore)*, **57**: 1736-1741, 1994.
5. Marre, F., Sanderink, G. J., de Sousa, G., Gaillard, C., Martinet, M., and Rahmani, R. Hepatic biotransformation of docetaxel (Taxotere) *in vitro*: involvement of the CYP3A subfamily in humans. *Cancer Res.*, **56**: 1296-1302, 1996.
6. De Valeriola, D., Brassinne, C., Gaillard, C., Ketter, J. P., Tomiak, E., Van Vreckem, A., Frühling, J., Frydman, A., Kerger, J., Piccart, M., Chapelle, P., and Blanc, C. Study of excretion balance, metabolism and protein binding of C^{14} radiolabeled Taxotere in cancer patients. *Proc. Am. Assoc. Cancer Res.*, **34**: 373, 1993.
7. Bruno, R., Vivier, N., Vergniol, J. C., DePhillips, S. L., Montay, G., and Sheiner, L. B. A population pharmacokinetic model for docetaxel (Taxotere): model building and validation. *J. Pharmacokinet. Biopharm.*, **24**: 153-172, 1996.
8. Bruno, R., Hille, D., Riva, A., Vivet, N., ten Bokkel Huinink, W. W., van Oosterom, A. T., Kaye, S. B., Verweij, J., Fossella, F. V., Valero, V., Rigas, J. R., Seidman, A. D., Chevallier, B., Fumoleau, P., Burris, H. A., Ravdin, P. M., and Sheiner, L. B. Population pharmacokinetics/pharmacodynamics of docetaxel in Phase II studies in patients with cancer. *J. Clin. Oncol.*, **16**: 187-196, 1998.
9. Kaye, S. B. Docetaxel (Taxotere) in the treatment of solid tumors other than breast and lung cancer. *Semin. Oncol.*, **22** (Suppl. 4): 30-33, 1995.
10. van Hoesel, Q. G., Verweij, J., Catimel, G., Clavel, M., Kerbrat, P., van Oosterom, A. T., Kerger, J., Tursz, T., van Glabbeke, M., and van Pottelsberghe, C. Phase II study with docetaxel (Taxotere) in advanced soft tissue sarcomas of the adult. *Ann. Oncol.*, **5**: 539-542, 1994.
11. Wagner, D. CYP3A4 and the erythromycin breath test. *Clin. Pharmacol. Ther.*, **64**: 129-130, 1998.
12. Baille, P., Bruno, R., Schellens, J. H., Webster, L. K., Millward, M., Verweij, J., and Montay, G. Optimal sampling strategies for Bayesian estimation of docetaxel (Taxotere) clearance. *Clin. Cancer Res.*, **3**: 1535-1538, 1997.
13. Vergniol, J. C., Bruno, R., Montay, G., and Frydman, A. Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. *J. Chromatogr.*, **582**: 273-278, 1992.
14. Beal, S. L., and Shener, L. B. (eds.), *Nonlinear Mixed Effects Model Users Guides*. San Francisco, CA: NONMEM Project Group, University of California at San Francisco, 1999.
15. Bruno, N., Vivier, C., Houver, G., Montay, G., and Riva, A. Lack of influence of dexamethasone premedication on docetaxel (Taxotere) pharmacokinetics. *Ann. Oncol.*, **7** (Suppl. 1): A335, 1996.

linization or acidification of the urine. Whether alteration of urine pH results in significant change in drug elimination depends upon the extent and persistence of the pH change and the contribution of pH-dependent passive reabsorption to total drug elimination. The effect is greatest for weak acids and bases with pK_a values in the range of urinary pH (5 to 8). However, alkalization of urine can produce a fourfold to sixfold increase in excretion of a relatively strong acid such as salicylate when urinary pH is changed from 6.4 to 8.0. The fraction of nonionized drug would decrease from 1% to 0.04%.

Biliary and Fecal Excretion. Many metabolites of drugs formed in the liver are excreted into the intestinal tract in the bile. These metabolites may be excreted in the feces; more commonly, they are reabsorbed into the blood and ultimately excreted in the urine. Both organic anions, including glucuronides, and organic cations are actively transported into bile by carrier systems similar to those that transport these substances across the renal tubule. Both transport systems are nonselective, and ions of like charge may compete for transport. Steroids and related substances are transported into bile by a third carrier system. The effectiveness of the liver as an excretory organ for glucuronide conjugates is very much limited by their enzymatic hydrolysis after the bile is mixed with the contents of the small intestine, and the parent drug can be reabsorbed from the intestine. Thus, such compounds may undergo extensive biliary cycling with eventual excretion by the kidney.

Excretion by Other Routes. Excretion of drugs into sweat, saliva, and tears is quantitatively unimportant. Elimination by these routes is dependent mainly upon diffusion of the nonionized, lipid-soluble form of drugs through the epithelial cells of the glands and is pH dependent. Reabsorption of the nonionized drug from the primary secretion probably also occurs in the ducts of the glands, and active secretion of drugs across the ducts of the gland may also occur. Drugs excreted in the saliva enter the mouth, where they are usually swallowed. The concentration of some drugs in saliva parallels that in plasma. Saliva may therefore be a useful biological fluid in which to determine drug concentrations when it is difficult or inconvenient to obtain blood.

The same principles apply to excretion of drugs in breast milk. Since milk is more acidic than plasma, basic compounds may be slightly concentrated in this fluid, and the concentration of acidic compounds in the milk is lower than in plasma. Nonelectrolytes, such as ethanol and urea, readily enter breast milk and reach the same concentration as in plasma, independent of the pH of the milk. (See Atkinson *et al.*, 1988.)

Although excretion into hair and skin is also quantitatively unimportant, sensitive methods of detection of toxic metals in these tissues have forensic significance. Arsenic in Napoleon's hair, detected 150 years after administration, has raised interesting questions about how he died, and by whose hand. Mozart's manic behavior during the preparation of his last major work, the *Requiem*, may have been due to mercury poisoning; traces of the metal have been found in his hair.

CLINICAL PHARMACOKINETICS

A fundamental hypothesis of clinical pharmacokinetics is that a relationship exists between the pharmacological or toxic response to a drug and the concentration of the drug in a readily accessible site in the body (*e.g.*, blood). This hypothesis has been documented for many drugs (see Appendix II), although it is apparent for some drugs that no clear or simple relationship has been found between pharmacological effect and concentration in plasma. In most cases, as depicted in Figure 1-1, the concentration of drug in the systemic circulation will be related to the concentration of drug at its sites of action. The pharmacological effect that results may be the clinical effect desired, a toxic effect, or, in some cases, an effect unrelated to efficacy or toxicity. Clinical pharmacokinetics attempts to provide both a more quantitative relationship between dose and effect and the framework with which to interpret measurements of concentrations of drugs in biological fluids. The importance of pharmacokinetics in patient care rests on the improvement in efficacy that can be attained by attention to its principles when dosage regimens are chosen and modified.

The various physiological and pathophysiological variables that dictate adjustment of dosage in individual patients often do so as a result of modification of pharmacokinetic parameters. The three most important parameters are *clearance*, a measure of the body's ability to eliminate drug;

volume of distribution, a measure of the apparent space in the body available to contain the drug; and *bioavailability*, the fraction of drug absorbed as such into the systemic circulation. Of lesser importance are the *rates* of availability and distribution of the agent.

CLEARANCE

Clearance is the most important concept to be considered when a rational regimen for long-term drug administration is to be designed. The clinician usually wants to maintain steady-state concentrations of a drug within a known therapeutic range (see Appendix II). Assuming complete bioavailability, the steady state will be achieved when the rate of drug elimination equals the rate of drug administration:

$$\text{Dosing rate} = CL \cdot C_{ss} \quad (1)$$

where CL is clearance and C_{ss} is the steady-state concentration of drug. Thus, if the desired steady-state concentration of drug in plasma or blood is known, the rate of clearance of drug by the patient will dictate the rate at which the drug should be administered.

The concept of clearance is extremely useful in clinical pharmacokinetics because clearance of a given drug is usually constant over the range of concentrations encountered clinically. This is true because systems for elimination of drugs are not usually saturated and, thus, the *absolute* rate of elimination of the drug is essentially a linear function of its concentration in plasma. A synonymous statement is that the elimination of most drugs follows first-order kinetics—a constant *fraction* of drug is eliminated per unit of time. If mechanisms for elimination of a given drug become saturated, the kinetics become zero-order—a constant *amount* of drug is eliminated per unit of time. Under such a circumstance, clearance becomes variable. Principles of drug clearance are similar to those of renal physiology, where, for example, creatinine clearance is defined as the rate of elimination of creatinine in the urine relative to its concentration in plasma. At the simplest level, clearance of a drug is the

rate of elimination by all routes normalized to the concentration of drug C in some biological fluid:

$$CL = \text{Rate of elimination}/C \quad (2)$$

It is important to note that clearance does not indicate how much drug is being removed but, rather, the volume of biological fluid such as blood or plasma that would have to be completely freed of drug to account for the elimination. Clearance is expressed as a volume per unit of time. Clearance is usually further defined as blood clearance (CL_b), plasma clearance (CL_p), or clearance based on the concentration of unbound or free drug (CL_u), depending on the concentration measured (C_b , C_p , or C_u). (For additional discussion of clearance concepts, see Benet *et al.*, 1984.)

Clearance by means of various organs of elimination is additive. Elimination of drug may occur as a result of processes that occur in the kidney, liver, and other organs. Division of the rate of elimination by each organ by a concentration of drug (e.g., plasma concentration) will yield the respective clearance by that organ. Added together, these separate clearances will equal total systemic clearance:

$$CL_{\text{renal}} + CL_{\text{hepatic}} + CL_{\text{other}} = CL_{\text{systemic}} \quad (3)$$

Other routes of elimination could include that in saliva or sweat, partition into the gut, and metabolism at other sites.

Total systemic clearance may be determined at steady state by using equation 1. For a single dose of a drug with complete bioavailability and first-order kinetics of elimination, total systemic clearance may be determined from mass balance and the integration of equation 2 over time:

$$CL = \text{Dose}/AUC \quad (4)$$

where AUC is the total area under the curve that describes the concentration of drug in the systemic circulation as a function of time (from zero to infinity).

Examples. In Appendix II, the plasma clearance for cephalexin is reported as $4.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, with 91% of the drug excreted unchanged in the urine. For a 70-kg man, the total body clearance from plasma would be 300 ml/min, with renal clearance accounting for 91% of this elimination. In other words, the kidney is able to excrete cephalexin at a rate such that approximately 273 ml of

Hepatic Biotransformation of Docetaxel (Taxotere®) *in Vitro*: Involvement of the CYP3A Subfamily in Humans¹

Françoise Marre,² Ger-Jan Sanderink,³ Georges de Sousa, Claude Gaillard, Michel Martinet, and Roger Rahmani³

Institut National de la Santé et de la Recherche Médicale, 41 Bd. du Cap, BP 2078, 06606 Antibes Cedex [F. M., G. d. S., R. R.], and Rhône-Poulenc Rorer SA Recherche-Development, Centre de Recherche de Vitry-Alfortville, 3 Digue d'Alfortville, 94140 Alfortville [G.-J. S., C. G., M. M.], France

ABSTRACT

Docetaxel metabolism mediated by cytochrome P450-dependent monooxygenases was evaluated in human liver microsomes and hepatocytes. In microsomes, the drug was converted into four major metabolites resulting from successive oxidations of the *tert*-butyl group on the synthetic side chain. Enzyme kinetics appeared to be biphasic with a V_{max} and apparent K_m for the high-affinity site of 9.2 pmol/min/mg and 1.1 μ M, respectively. The intrinsic metabolic clearance in human liver microsomes (V_{max}/K_m , 8.4 ml/min/g protein) was comparable to that in rat and dog liver microsomes, but lower than in mouse liver microsomes.

Although the metabolic profile was identical in all subjects, a large quantitative variation in docetaxel biotransformation rates was found in a human liver microsome library, with a ratio of 8.9 in the highest:lowest biotransformation rates. Docetaxel biotransformation was correlated significantly (0.7698; $P < 0.0001$) with erythromycin *N*-demethylase activity, but not with aniline hydroxylase or debrisoquine 4-hydroxylase. It was inhibited, both in human hepatocytes and in liver microsomes, by typical CYP3A substrates and/or inhibitors such as erythromycin, ketoconazole, nifedipine, midazolam, and troleandomycin. Docetaxel metabolism was induced *in vitro* in human hepatocytes by dexamethasone and rifampicin, both classical CYP3A inducers. These data suggest a major role of liver cytochrome P450 isoenzymes of the CYP3A subfamily in docetaxel biotransformation in humans. Finally, some *Vinca* alkaloids and doxorubicin were shown to inhibit docetaxel metabolism in human hepatocytes and liver microsomes. These findings may have clinical implications and should be taken into account in the design of combination cancer chemotherapy regimens.

INTRODUCTION

Docetaxel (Taxotere®, RP 56976; Fig. 1), a semisynthetic taxoid, is an antimetabolic agent belonging to the spindle poison family (1). The drug promotes the assembly of stable microtubules *in vitro* and is an inhibitor of cell replication (2). The compound has demonstrated excellent antitumor activity against various types of solid tumors in preclinical models (3, 4) and is currently undergoing Phase II and III clinical evaluation. The pharmacokinetics of docetaxel has been studied after i.v. infusion in mice, rat, dogs, and cancer patients and showed multiphasic disposition profiles with rapid initial tissue uptake and large volumes of distribution. In these species, the drug is eliminated almost exclusively by hepatic metabolism and biliary excretion, with quite similar metabolic profiles. The parent drug is the main circulating compound, and metabolites of docetaxel described thus far are much less cytotoxic (4-8).

Metabolism represents a major factor of variability in drug response and toxicity particularly for anticancer compounds that exhibit a narrow therapeutic index. Among the various hepatic enzyme systems

involved in the metabolism of drugs, the CYP⁴ superenzyme family was recognized early as the chief contributor. Currently, at least 15 human liver CYPs involved in drug metabolism have been characterized in terms of molecular, spectral, enzymatic, and immunological properties (9). This multigene superfamily of hemoproteins is involved in the deactivation and/or activation of numerous endogenous substances (e.g., steroids and fatty acids) and xenobiotics (e.g., drugs, carcinogens). CYPs are involved directly in a number of drug-induced hepatotoxicities and drug interactions. Some of these adverse effects are caused by genetic polymorphism. Others result either from drug interactions occurring between several coadministered drugs that are metabolized by the same CYP, or from the specific induction of the CYP in question. Thus, understanding the role of the CYPs involved in a given drug's metabolism and their regulation will be useful in determining the range of interindividual variability in drug response and avoiding undesirable drug interactions. This is a matter of particular concern in cancer chemotherapy, in which many prescriptions may be encountered simultaneously. Therefore, since most anticancer drugs produce a number of side effects, a better understanding of the enzyme systems that process these compounds would be of great use in designing optimal therapy protocols.

To date, research on the identification of specific CYP isozymes in the metabolism of cancer chemotherapeutic agents has been limited to a few compounds such as CYCLO or procarbazine, where rat hepatic microsomes were used (10). Except for some very recent studies on *Vinca* alkaloids and paclitaxel (11-13), little data on the human enzymes involved in the metabolism of cancer chemotherapeutic agents are available. In that context, we evaluated the biotransformation of docetaxel by using microsomes and/or hepatocytes from human livers. The aim of the present report was to identify hepatic CYP isozymes involved in docetaxel biotransformation in humans and to evaluate possible metabolic drug interactions between docetaxel and other anticancer agents, which are potentially associated with this taxoid.

MATERIALS AND METHODS

Chemicals. Docetaxel (Taxotere®, RP56976, batch PHI2512), DZP, IMI, INTO (RP60475), PB, RANI, and DOXO were obtained from the Centre de Recherche of Rhône-Poulenc Rorer SA (Vitry-sur-Seine, France). [¹⁴C]Docetaxel (specific activity, 50 mCi/mmol) was synthesized by the Centre des Etudes Nucléaires (Gif-sur-Yvette, France). α -NF, β -NF, ANI, BP, CIME, DEX, ERY, KETO, NADPH, NIF, propranolol, QUI, SPA, TP, TB, and TAO were purchased from Sigma (Saint Quentin Fallavier, France). DBQ, MDZ, and SKF525A (proadifen) were a gracious gift from Hoffmann LaRoche (Basel, Switzerland). ACET and QUIS were obtained from E. Merck; CAF

Received 5/2/95; accepted 1/15/96.

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¹ This work was supported in part by a grant from Ligue Nationale Contre le Cancer.

² Present address: Laboratoire de Biochimie, Faculté de Pharmacie, 2 rue du Dr Raymond Marcland, 87025 Limoges Cedex, France.

³ To whom requests for reprints should be addressed.

⁴ The abbreviations used are: CYP, cytochrome P450; DZP, diazepam; IMI, imipramine; INTO, intoplicine; PB, phenobarbital; RANI, ranitidine; DOXO, doxorubicin; α -NF, α -naphthoflavone; β -NF, β -naphthoflavone; ANI, aniline; BP, benzo[a]pyrene; CIME, cimetidine; DEX, dexamethasone; ERY, erythromycin; KETO, ketoconazole; NIF, nifedipine; QUI, quinidine; SPA, sparteine; TP, theophylline; TB, tolbutamide; TAO, troleandomycin; DBQ, debrisoquine; 3MC, 3-methylcholanthrene; MDZ, midazolam; ACET, acetanilide; VP16, etoposide; QUIS, quinine sulfate; CAF, caffeine; CISPT, cisplatin; CYCLO, cyclophosphamide; 5-FU, 5-fluorouracil; HB, hexobarbital; VRB, vinorelbine; OME, omeprazole; IC₅₀, 50% inhibitory concentration; VCR, vincristine; VBL, vinblastine; HPLC, high-performance liquid chromatography; PARA, paracetamol; HBSS, Hank's balanced salt solution.

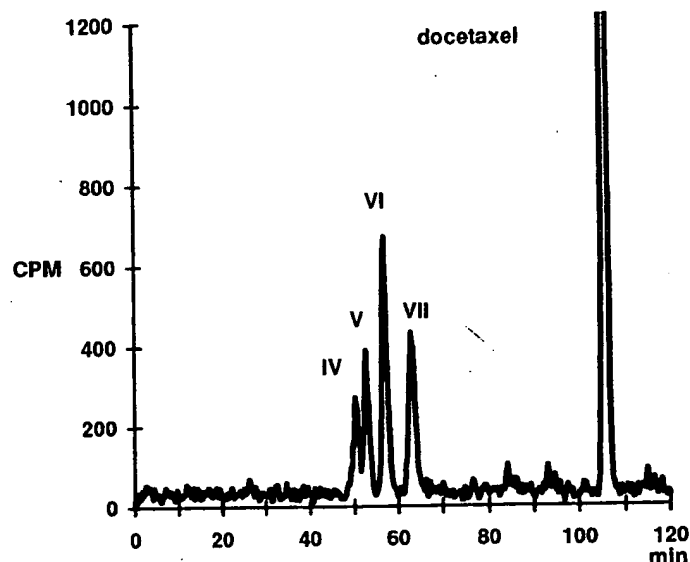


Fig. 1. HPLC metabolic pattern of docetaxel in liver microsomes. Incubation time, 60 min; on-line radioactivity detection. VI, alcohol derivative; V and VII, cyclized aldehyde derivatives; IV, carboxylic acid derivative.

from Prolabo; CISPT from Roger Bellon; CYCLO from Sarget; 5-FU from Roche; HB from Boehringer; VRB from Pierre Fabre, OME from Research Biochemicals, Inc.; VP16 from Sandoz; and VCR, and VBL from Eli Lilly. Other chemicals were purchased from commercial sources and were of analytical or HPLC grade.

Preparation and Use of Liver Microsomal Fractions. Liver microsomes were prepared from pools of livers from 6-week-old male OF1 mice (23–28 g) and OFA Sprague-Dawley rats (180–200 g; IFFA-CREDO, Saint Germain-sur-l'Arbresle, France) and a liver from a male beagle dog (10 kg; Shamrock Farms, United Kingdom). Animals were maintained under standardized conditions of light and temperature. They had free access to food and water. Food was withdrawn 16 h before sacrifice. Human liver portions were from organ transplant donors or normal fractions of operative specimens obtained from hepatic segmentectomy of liver tumors. Characteristics of donors were reported previously (12). Microsomal fractions were prepared as described previously (14). Microsomal protein concentration was determined according to the method of Bradford (Bio-Rad Protein Assay kit; Ref. 15) using bovine serum albumin as standard. Concentrations of CYP and cytochrome b5 were measured according to Omura and Sato (16). The incubation mixtures contained microsomes (2 mg microsomal protein/ml) and NADPH (1 mM) in a potassium phosphate buffer (100 mM, pH 7.4). Incubations were carried out at 37°C in a shaking water bath under atmospheric oxygen. Reactions were initiated by the addition of pure or isotopic dilutions of [¹⁴C]docetaxel (50 mCi/mmol) and stopped by 1 volume of methanol. The samples were centrifuged (10,000 × g, 2 min), and supernatant fluids were analyzed using HPLC without further processing. Each experiment was performed in triplicate.

Incubations for analysis of enzyme kinetics were carried out at docetaxel concentrations from 2 to 100 μM, with sampling at several time points between 0.5 and 60 min. The kinetics of the biotransformation of the substrate was monitored. This was more appropriate than analysis of metabolites because only one initial oxidation reaction occurred on the parent drug, but the primary metabolite was further oxidized (see "Results").

For interindividual variability studies in human microsomes, metabolism of docetaxel (5 μM) was measured in the microsomal fractions (n = 29) incubated for 30 min. Incubations for inhibition studies with human liver microsomes were carried out in triplicate for 60 min at a target concentration of 5 μM docetaxel. The CYP substrates or inhibitors used were ACET, α-NF, CAF, TB, QUI, QUIS, ANI, ERY, KETO, NIF, TAO, and SKF525A. Concentrations ranged from 1 to 1000 μM (Table 2). In each experiment, control incubations were included containing the appropriate vehicle, and reference incubations in which the substrate was added after enzyme inactivation with methanol. In case of inhibition, IC₅₀ values were determined. Moreover, for KETO, kinetic

studies were performed to determine apparent *K_i* values; microsomal fractions were incubated for 30 min with 1, 2.5, and 5 μM docetaxel and 0.5, 1, 2, 5, and 10 μM KETO.

Human liver microsomal fractions were also characterized by using specific substrates of several CYP isoforms. ERY *N*-demethylase activity was determined by colorimetric estimation of the formaldehyde formed after a 20-min incubation period of microsomal fractions (1 mg microsomal protein/ml) with 2 mM ERY (17). ANI hydroxylase and DBQ 4-hydroxylase activities were determined as described previously (18, 19).

Hepatocyte Isolation and Use of Primary Culture. A discarded human hepatic tissue fragment from a patient with secondary hepatic tumor (IU 46) and normal hepatic fragments (HL 45, 50, and 51) were obtained from the Sainte Marguerite Hospital (Professor P. Fuentes and Dr. P. Thomas, Marseille, France). After the tissue was washed with HBSS, hepatocytes were isolated by conventional collagenase perfusion as described previously (20). Using such a method, viability of cells, determined by erythrosine B exclusion test, was at least 80%.

After washing (three times) with L15 medium, cells were resuspended in William's E medium supplemented with FCS (10%), insulin (0.1 IU/ml), penicillin (50 IU/ml), streptomycin (50 μg/ml), and netilmicine (50 μg/ml). Hepatocytes were seeded in 6-well (800,000 cells/well) and 12-well (300,000 cells/well) plates precoated with collagen type I (200 and 100 μg/well, respectively). Four hours later, the medium was renewed.

To screen for potential inducers of docetaxel metabolism, the medium was removed 24 h after seeding and replaced by William's E medium containing insulin, penicillin, streptomycin, netilmicine, and 5% FCS. Hepatocytes were maintained in primary culture for 72 h in the presence of each of the following inducers: PB (2 mM), 3MC (40 μM), β-NF (50 μM), DEX (50 μM), and RIF (50 μM). Medium was then removed and replaced by HBSS without inducer and containing [¹⁴C]docetaxel. After 24 h of exposure, the medium was sampled and cells were scraped with water/methanol/hydrochloric acid (v/v, 0.1 N). The samples were analyzed using HPLC without further processing.

To evaluate the potential inhibitory effect of characteristic substrates, cells were preincubated with RIF as described above. After a 72-h incubation period, the medium was replaced by HBSS containing 5 μM [¹⁴C]docetaxel. Cells were incubated in the absence or presence of 5 or 25 μM of each of the following compounds: BP, TP, HB, DBQ, SPA, ANI, ERY, NIF, KETO, MDZ, QUI, CIME, and SKF525A. Cells were then incubated under the usual conditions.

Metabolic Drug Interactions. A number of anticancer agents and other drugs potentially associated with docetaxel in cancer chemotherapy were assessed for their inhibitory effects on docetaxel biotransformation. These drugs included ARAC, CISPT, CYCLO, 5-FU, DOXO, INTO, VP16, VCR, VBL, and VRB as anticancer agents and CIME, DZP, IMI, OME, PARA, and RANI as other drugs. Assays were carried out using HL 50- and HL 51-cultured hepatocytes and human microsomal fractions in the optimal conditions described above. Final docetaxel concentration in the incubation medium was 5 μM; the drugs were used at different concentrations that ranged between 0.25 and 1000 μM. All assays were done in triplicate.

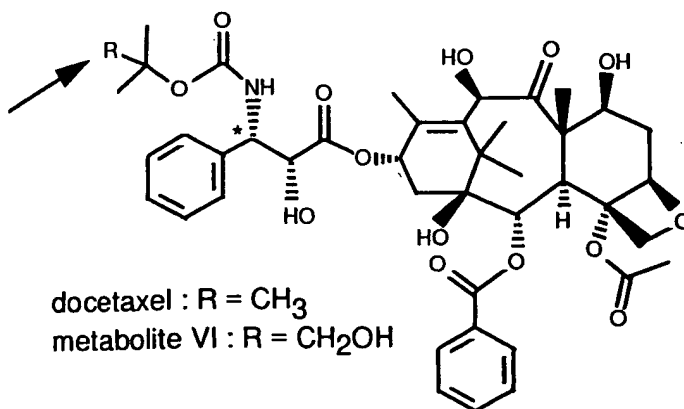


Fig. 2. Structure of docetaxel and its main metabolite. *, site of ¹⁴C radiolabel. Arrow, position of metabolic oxidation.

Table 1 Enzyme kinetics constants of docetaxel metabolism in liver microsomes

Microsomal fractions (1 mg microsomal protein/ml) were incubated at 37°C with docetaxel (range, 2–100 μM) for 0.5–60 min. Reactions were initiated by the addition of NADPH (1 mM) and stopped by methanol/hydrochloric acid (v/v, 0.1 N) before HPLC analysis.

Species	V_{\max} (pmol/min/mg protein)	K_m (μM)	Clm^a (ml/min/g protein)
Mouse	258	10.3	25
Rat ^b	22.2	6.4	3.5
Dog	43.5	8.5	5.1
Human ^b	9.2	1.1	8.4

^a Intrinsic metabolic clearance (V_{\max}/K_m).

^b Parameters of the first term of a two-enzyme model.

HPLC Analysis. HPLC analysis for kinetic studies was performed with a Waters model 510 equipped with an automatic Waters 715 UltraWisp injector. Separation was performed on an Ultrasphere ODS Beckman column (5 μm , 4.6 \times 250 mm). Elution was carried out at 1 ml/min by using a water:methanol (v/v, 35:65) mixture, delivered isocratically. Radiolabeled compounds were detected by continuous flow liquid scintillation (Flow-One; Packard). Under the above conditions, the retention time for docetaxel was 22.5 min.

Docetaxel metabolic profiles were analyzed with a high-pressure gradient system consisting of Merck L6200 and L6000 pumps, a Waters 717 UltraWisp injector, a Merck L4000 UV detector, and a Berthold LB 506C continuous flow scintillation detector. Docetaxel and its metabolites were separated on a Merck RP Select B column (5 μm , 4 \times 250 mm) thermostated at 25°C. The mobile phase solutions consisted of water:acetic acid:acetonitrile in the proportions 95:0.2:5 (solution A) or 5:0.2:95 (solution B). The gradient was 26% B (0–60 min), 26–35% B (60–80 min), 35% B (80–105 min), 35–100% B (105–115 min), and 100% B (115–125 min). Flow rate was 1 ml/min.

The detection limit of the methods was 150 dpm (three times background counts), which corresponded in routine incubations to $<0.05 \mu\text{M}$. The repeatability of triplicate incubation assays was about 5%.

Data Analysis. Biotransformation rates and enzyme kinetic parameters were calculated using iterative nonlinear regression analysis with the program Enzfitter (Elsevier-BIOSOFT, Cambridge, United Kingdom). IC_{50} concentrations were calculated from linearized plots of rate^{-1} against inhibitor concentration.

Correlation between the enzyme activities and docetaxel metabolism rate was determined using linear regression analysis on MicroStat PC software. Variance analysis (F test) was performed to validate the linear model hypothesis and correlation was considered significant when the null hypothesis (slope = constant) could be rejected at the 5% risk level.

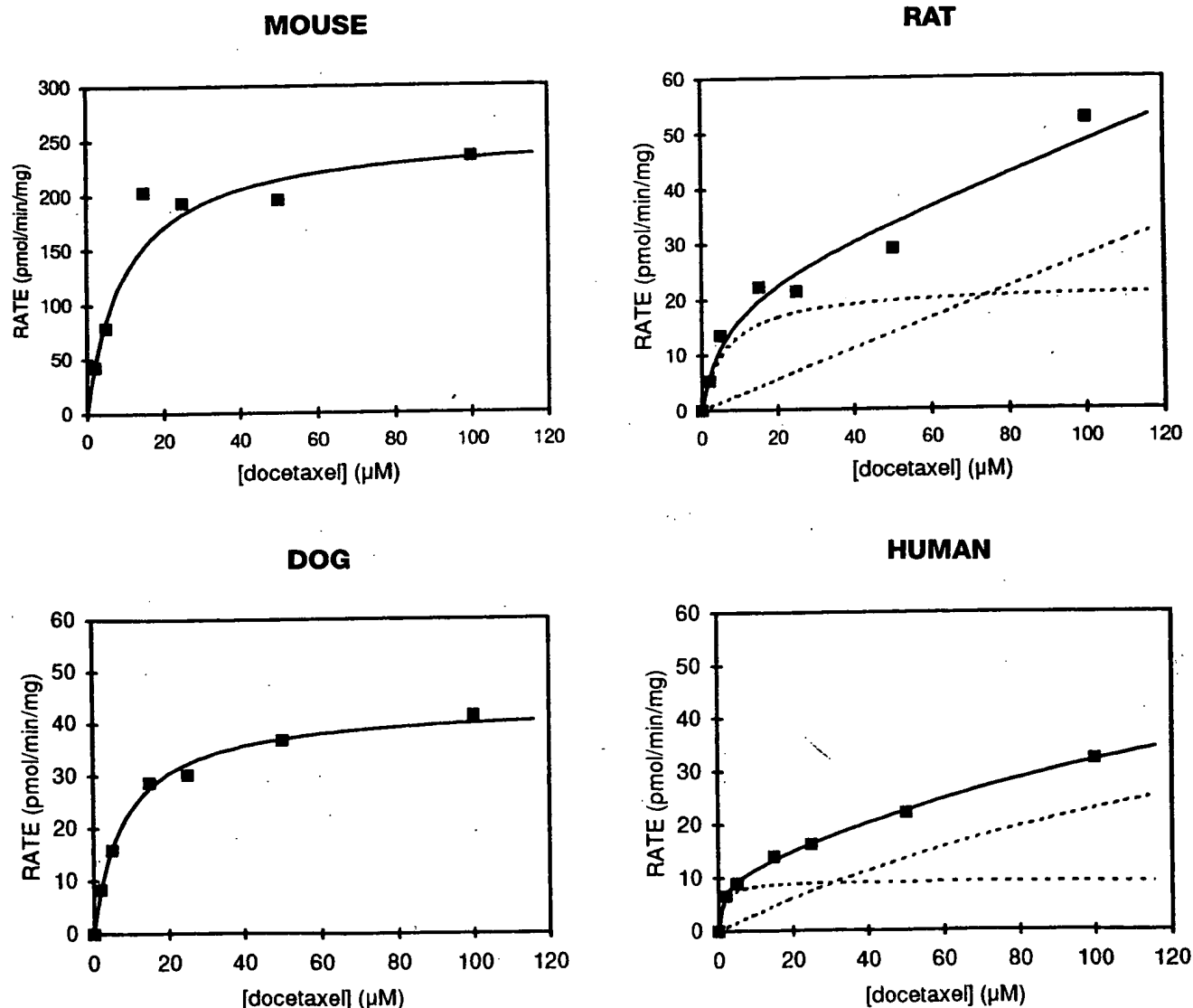


Fig. 3. Interspecies variability of docetaxel metabolism: determination of Michaelis-Menten constants. Concentration-rate data were fitted to a one-enzyme model for mouse and dog liver microsomes and to a two-enzyme model for rat and human liver microsomes. ---, curves for the high- and low-affinity sites of these latter species. [Left curves (rapidly increasing at the beginning and leveling off first), high-affinity sites; right curves, low-affinity sites.]

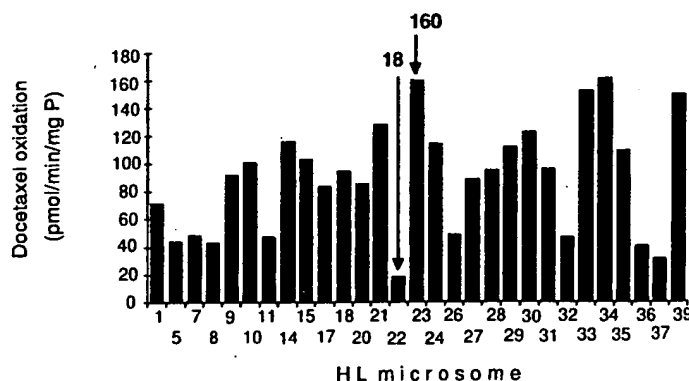


Fig. 4. Interindividual variability of docetaxel oxidation in human liver microsomes. Human liver microsomal fractions (1 mg microsomal protein/ml) were incubated at 37°C for 30 min with docetaxel (5 μ M).

RESULTS

Metabolic Pattern of Docetaxel in Liver Subcellular Fractions. The metabolic pattern of docetaxel obtained after a 60-min incubation with human liver microsomes in the presence of NADPH is illustrated in Fig. 1. Formation of four metabolites, each more polar than the parent drug, was observed. The metabolites were identified by comparison with metabolites isolated from human feces by Gaillard and colleagues (6–8) and synthesized by Commerçon *et al.* (21). All *in vitro* metabolites resulted from successive oxidations of the *tert*-butyl group on the synthetic side chain (Fig. 2). The major metabolite (VI) corresponded to the alcohol. Metabolites V and VII are two oxazolidine-type compounds, resulting from cyclization of an unstable intermediate aldehyde. Metabolite IV corresponds to the carboxylic acid. Following cyclization this compound may yield an oxazolidinedione derivative, the major docetaxel metabolite in human feces (7).

Interspecies and Interindividual Variability of Docetaxel Biotransformation Rate. Docetaxel was incubated at concentrations from 2 to 100 μ M with mouse, rat, dog and human liver microsomes in the presence of NADPH. Enzyme kinetic parameters show that biotransformation rates were similar in the rat, dog, and human liver microsomes, but considerably higher in mouse liver microsomes (Table 1). These rates are also shown in Fig. 3, with the best-fit curves calculated according to the Michaelis-Menten equation. For mouse and dog liver microsomes, the best fit was observed with the classical equation for one enzyme site. For rat and human liver microsomes, a two-enzyme model appeared to be more appropriate. The parameters for the low-affinity site in rat and human liver microsomes are not included because saturation was insufficient in the experimental range of substrate concentrations.

Docetaxel biotransformation rates were studied in 29 human liver microsomal samples. Results (Fig. 4) indicate a significant quantitative variability among individuals since docetaxel (5 μ M) biotransformation rates ranged between 18 and 160 nmol total metabolites/min/mg microsomal protein, while the mean rate was 89.5 nmol total metabolites/min/mg microsomal protein (coefficient of variation, 44.7%).

Inhibition of Docetaxel Metabolism. The effect of several CYP inhibitors on docetaxel biotransformation in human liver microsomes is shown in Table 2. Docetaxel metabolism in human liver microsomes was decreased most effectively by the CYP3A substrates and/or inhibitors ERY, KETO, and NIF. Among these compounds, KETO exhibited an IC_{50} value of about 1 μ M, TAO about 10 μ M, and NIF about 130 μ M. The apparent K_i value for KETO was 1.35 μ M. The CYP1A2-inhibitor α -NF and substrates CAF and ACET, the CYP2C8/9 substrate TB, the CYP2D6 inhibitor QUI, and the CYP2E

substrate ANI had no effect. The nonspecific suicide substrate SKF525A reduced docetaxel biotransformation by 40%.

Human hepatocytes from HL 45 and HL 46 were incubated for 24 h in the presence of 5 μ M docetaxel and 5 or 25 μ M of each potential inhibitor. The results (Table 3) confirm that only specific substrates or inhibitors of the CYP3A subfamily (KETO, NIF, and MDZ) inhibit the biotransformation of docetaxel to a significant extent.

Induction of Docetaxel Metabolism. Human hepatocytes from HL 45 and HL 46 were preincubated for 72 h with the classical inducers PB, 3MC, β -NF, and RIF. Docetaxel (5 μ M) metabolism was evaluated subsequently in these induced hepatocytes.

Table 3 illustrates that the biotransformation of docetaxel was increased by the pretreatment of hepatocytes with RIF (+44% compared to control) and DEX (+41%), whereas PB (+8%), β -NF (+20%), and 3MC (0%) had less or no effect.

Correlations with Monooxygenase Activities. An approach that has been used frequently to associate activities to a specific enzyme in human liver is to correlate the activity of a selected substrate toward that of known substrate in a variety of human liver samples. We used a bank of 25 human liver microsomal samples that had been phenotyped previously with respect to the relative level of various CYP-

Table 2 Effects of CYP substrates/inhibitors on docetaxel (5 μ M) metabolism by human liver microsomes

Microsomal fractions (2 mg protein/ml) were preincubated for 10 min with various potential inhibitors at 37°C before incubation with docetaxel (5 μ M) for 60 min.

Compound	Main CYP isoenzyme affinity	Concentration (μ M)	Inhibition (%)	IC_{50} (μ M)
ACET	1A2	1000	13	— ^a
α -NF	1A2	1	—1	—
CAF	1A2	400	23	—
TB	2C8/9	50	—7	—
QUI	2D6	5	—7	—
QUIS	(2D6 control)	5	7	—
ANI	2E1/2	50	18	—
ERY	3A3/4	1000	53	—
KETO	3A3/4	10	99	1 (0.1–10) ^b
NIF	3A3/4	100	44	130 (30–300) ^b
TAO	3A3/4	10	53	10 (1–300) ^b
SKF525A	(nonspecific)	250	40	—

^a —, not determined.

^b Concentration range of compounds used to determine IC_{50} values.

Table 3 Effects of inhibitory and inducer compounds on docetaxel (5 μ M) metabolism by human hepatocytes in primary culture

Cultured human hepatocytes were incubated either with enzyme inducers (PB, 3MC, β -NF, DEX, and RIF) for 72 h and then with docetaxel (5 μ M) for 24 h; or with potential inhibitors (BP, TP, HB, DBQ, SPA, ANI, ERY, NF, KETO, MDZ, QUI, and SKF525A) and docetaxel (5 μ M) simultaneously for 24 h.

Compound	Concentration (μ M)	Inhibition (%)	Induction (%)
3MC	40	—	0
BP	5	26	— ^a
β -NF	50	—	+20
TP	5	29	—
PB	2000	—	+8
HB	5	24	—
DBQ	5	24	—
SPA	5	27	—
QUI	5	44	—
ANI	5	24	—
RIF	50	—	+44
DEX	50	—	+41
ERY	5	38	—
KETO	5	95	—
NIF	5	90	—
MDZ	5	77	—
SKF525A	5	44	—

^a —, not determined.

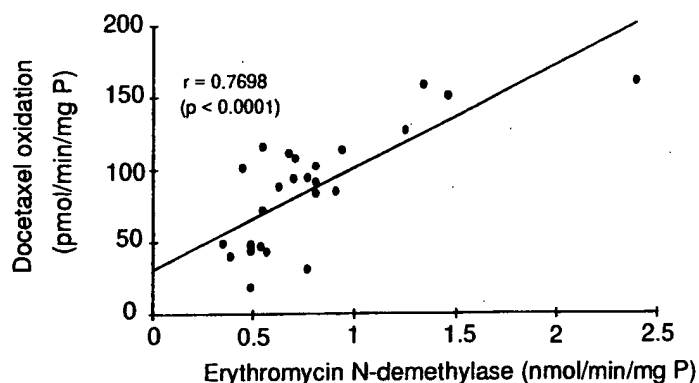


Fig. 5. Relationship between docetaxel oxidation and ERY *N*-demethylase activity. Human liver microsomal fractions (1 mg microsomal protein/ml) were incubated at 37°C with docetaxel (5 μ M, 30 min) or ERY (2 mM, 20 min). *r* was determined using linear regression analysis.

specific monooxygenase activities: ERY *N*-demethylation (CYP3A subfamily), ANI hydroxylase (CYP2E subfamily), and DBQ 4-hydroxylase (CYP2D6). The only significant correlation obtained was the biotransformation of docetaxel with ERY *N*-demethylase activity ($r = 0.7698$, $P < 0.0001$), indicating that variability in docetaxel metabolism is likely caused by that in CYP3A levels (Fig. 5). In contrast, there was no correlation with either DBQ 4-hydroxylase ($r = -0.3373$, not significant) or ANI ($r = 0.3535$, not significant) activities measured in the same samples.

Metabolic Drug Interactions. Results of inhibition studies with various drugs are given in Table 4. DOXO, VRB, and VBL were able to inhibit docetaxel biotransformation *in vitro* with IC_{50} values of about 20, 60, and >100 μ M, respectively. The anticancer agents 5-FU, CYCLO, CISPT, ARAC, VCR, VP16, and INTO and the anti- H_2 drugs CIME, RANI, as well as DZP, IMI, and PARA had no effect, even at high concentrations. For OME, weak inhibition occurred at the highest tested concentration (100 μ M).

Cultured human hepatocytes were incubated in the presence of docetaxel (5 μ M) and each anticancer drug (0.25–5 μ M). Results (data not shown) were similar to those obtained with microsomal fractions.

DISCUSSION

The biotransformation of docetaxel was investigated in human hepatic *in vitro* models. Enzyme kinetics was compared with mouse, rat, and dog liver microsomes. Because previous studies did not indicate any direct conjugation reactions of docetaxel by rat and human UDP-glucuronosyltransferases or glutathione *S*-transferases,⁵ this study focused on docetaxel biotransformation by the CYP-dependent monooxygenase system. Docetaxel biotransformation rates were more rapid in mouse liver microsomes than in those from other species. Apparent K_m values were in the low micromolar range for all species. This finding appears to be in agreement with that of hepatic elimination of docetaxel in the isolated perfused rat liver being concentration dependent in the 5–50 μ M concentration range (22). The metabolism of docetaxel followed a one-model Michaelis-Menten kinetic pattern in mouse and dog liver microsomes and a two-model kinetic pattern in rat and human samples. The biphasic linear kinetics suggested a multienzyme binding site for docetaxel.

Docetaxel biotransformation by human liver microsomes showed a large quantitative interindividual variation. A variability factor of 9 was found between the lowest and the highest metabolic rates. This variability could be explained by the involvement of one or more

CYPs in docetaxel metabolism subject to variable regulation and/or genetic polymorphism. This study shows that isoenzymes of the CYP subfamily CYP3A appear to be the major forms responsible for docetaxel biotransformation in humans. Docetaxel metabolism by human cultured hepatocytes was induced by RIF and DEX. The CYP3A substrates and inhibitors TAO, ERY, and NIF (23, 24) reduced docetaxel biotransformation in human hepatocytes and liver microsomes. KETO, which had the same effect, is also known to be a very potent inhibitor of CYP3A (25). Moreover, the docetaxel metabolism rate was correlated strongly with ERY *N*-demethylase activity, a marker of CYP3A3/4 activity, but not with ANI hydroxylase (CYP2E1/2) or DBQ hydroxylase (CYP2D6). This shows that the interindividual variability in docetaxel oxidation is related to variability in expression of the CYP3A subfamily. Indeed, the expression of CYP3A4 is known to be highly variable in human liver and intestine, and the CYP3A5 isoform is thought to be polymorphic (24, 26). It is interesting that the main metabolizing enzyme of docetaxel is different from that of paclitaxel, which is transformed mainly by CYP2C8/9 in human liver microsomes (13).

This study provides some contradictory indications concerning the number of isoenzymes involved in docetaxel metabolism. On the one hand, the biotransformation kinetics of docetaxel in rat and human liver microsomes was most consistent with a two-enzyme model. The lack of a zero interception in the correlation with ERY *N*-demethylase may indicate that other minor CYP forms are involved in the metabolism of docetaxel. On the other hand, none of the inhibitors of other CYPs had a significant effect on docetaxel biotransformation in human liver microsomes. However, biphasic kinetics in human liver microsomes has been observed for other CYP3A substrates, and in one case evidence for substrate activation at high concentrations was given (27, 28). On the other side, the inhibition experiments in this study were carried out at a docetaxel concentration (5 μ M), at which almost all activity is catalyzed by the high-affinity site (Fig. 3). This may explain the absence of inhibition by compounds with affinity for other CYPs.

Among the tested inhibitors, QUI is a special case. This compound is a very potent inhibitor of CYP2D6 ($K_i < 1$ μ M), but metabolized itself mainly by CYP3A with an apparent K_m of about 30 μ M (29, 30). In our experiments the QUI concentration was 5 μ M; therefore, only an effect on CYP2D6 would be observable. As this was not the case, and as no difference between QUI and quinine (which is a much less

Table 4 Effects of drugs on docetaxel (5 μ M) metabolism by human liver microsomes. Microsomal fractions (2 mg protein/ml) were preincubated for 10 min with various drugs at 37°C before incubation with docetaxel (5 μ M) for 60 min.

Drug	Inhibition ^a (%)	IC_{50} ^b (μ M)
ARAC	-13	— ^c
5-FU	-12	—
CISPT	-8	—
CYCLO	-2	—
DOXO	86	20
INTO	5	—
VBL	44	>100
VCR	5	—
VP16	-29	—
VRB	63	60
CIME	8	—
RANI	11	—
OME	32	—
DZP	9	—
IMI	-26	—
PARA	-33	—

^a Inhibitor concentration, 100 μ M.

^b Concentration range, 1–1000 μ M.

^c —, not determined.

⁵ Unpublished data.

potent inhibitor of CYP2D6) was found, no influence of the DBQ phenotype on docetaxel metabolism is expected in humans.

So, taken together, docetaxel appears to be metabolized mainly by CYP3A isoenzyme(s), especially at therapeutic concentrations, which are 5 μM or lower (5). Although the involvement of other isoenzymes at high docetaxel concentrations is not to be excluded, this study gives no definitive proof for this. The CYP3A family of CYP is thought to be the predominant family in human drug metabolism. It contains four very closely related proteins (CYP3A3, CYP3A4, CYP3A5, and CYP3A7) which account for up to 60% of the total CYP expressed in human liver. CYP3A5 is expressed polymorphically, having been detected in only about 10–20% of adult human livers analyzed, whereas CYP3A7 is the major CYP expressed in the human fetal liver. The most important hepatic isoenzymes in adults are CYP3A3 and CYP3A4, having only 11 amino acid substitutions, and no differences in catalytic activities have been demonstrated; thus, they are commonly referred to as CYP3A3/4. They represent key enzymes responsible for the metabolism of many clinically important drug families like corticosteroids, antifungals, antineoplastic agents, immunosuppressants, macrolide antibiotics, sex hormones, opioid analgesics, antiarrhythmic agents, vasodilators, etc.

ERY *N*-demethylation, cyclosporin metabolism, NIF oxidation, MDZ hydroxylation, testosterone 6 β -hydroxylation, and cortisol 6 β -hydroxylation are probes of CYP3A3/4 catalytic activity. The levels of CYP3A3/4 vary highly among liver microsomal samples. Several drugs (cortisol, DEX, RIF, PB, and carbamazepine) are inducers of CYP3A, some of which are likely to be coadministered in patients. This regulation is not yet elucidated completely, but occurs probably at the level of transcription and does not involve a known steroid receptor. In addition, several potent inhibitors of 3A-mediated metabolism *in vivo* have been characterized (e.g., KETO, verapamil, ethinyl estradiol, TAO, gestodene, and narengenin).

Combination chemotherapy has become standard in the treatment of most malignancies due to a number of theoretical advantages and proven superior clinical efficacy compared to single-agent treatment. Protocols with three, four, or more drugs have been used in a variety of combinations, administered concurrently or sequentially. Yet, many unrecognized drug interactions undoubtedly occur. Therefore, evaluation of interactions between anticancer drugs are of considerable interest with regard to the optimization of cancer chemotherapy. Some drugs which may potentially be coadministered with docetaxel were tested for their capacity to modify docetaxel metabolism in human liver microsomes. For most of these drugs the interaction with specific human CYP isoenzymes has not been studied previously. The role of CYP3A in the metabolism of some *Vinca* alkaloids was demonstrated recently (11, 12). As for the *Vinca* alkaloids, an absence of inhibition of CYP3A-mediated metabolism by 5-FU, cytarabine, and cisplatin was observed for docetaxel. Inversely, the metabolism of docetaxel in human liver microsomes can also be inhibited by DOXO, VBL, and VRB. DOXO can be metabolized by NADPH-CYP reductase (31), which may thus constitute an alternative inhibition site, along with the CYP3A enzymes. Only the effects of VCR and VP16 diverged, both compounds being inhibitors of vindesine and VBL metabolism but not of docetaxel.

Among the other tested drugs, none except the already mentioned CYP3A substrates/inhibitors were found to inhibit docetaxel biotransformation. These results are consistent with the fact that in humans diazepam and OME are metabolized mainly by CYP2C19 (32, 33), imipramine by CYP2D6 and CYP1A2 (34, 35), and PARA by several enzymes, among them mainly CYP1A2 and CYP2E1 (36). The lack of inhibition by CIME is consistent with the absence of an effect on another human CYP3A-mediated reaction, cyclosporin oxidation (37).

Whether the metabolic drug-drug interactions observed in these *in*

vitro experiments will occur *in vivo* is likely to depend on the dosage and pharmacokinetics of both docetaxel and the coadministered drug(s). However, the concentrations in the *in vitro* models cannot be compared directly to *in vivo* plasma concentrations since factors such as hepatic uptake or protein binding will be of importance. Concerning docetaxel, its low biotransformation rate *in vitro* is consistent with a rather low hepatic extraction rate *in vivo* (38). Its elimination is thus likely to be dependent on drug-metabolizing enzyme levels and could, in consequence, be affected by inhibitors and inducers. On the other hand, the low $K_{m(\text{app})}$ of 1.1 μM indicates a high affinity, which makes docetaxel metabolism less susceptible to competitive inhibition.

Our data demonstrate clearly the involvement of liver CYP3A isozymes in the biotransformation of docetaxel and significant metabolic drug interactions between this drug and a number of anticancer agents which could potentially be associated with docetaxel. Because inhibition of docetaxel metabolism may result in higher effective exposure of patients, and, regarding the low safety margin of this class of drugs, these findings may have both fundamental and clinical implications and should be taken into account in the evaluation and design of combination cancer chemotherapy regimens.

ACKNOWLEDGMENTS

We are grateful to A. Touzet for technical assistance. Special thanks are due to the clinical staff of the organ transplant units (Marseille, France).

REFERENCES

- Gueritte-Voegelein, F., Guenard, D., Lavelle, F., Le Goff, M. T., Mangatal, L., and Potier, P. Relationships between the structure of Taxol analogues and their metabolic activity. *J. Med. Chem.*, 34: 992–998, 1991.
- Ringel, I., and Horwitz, S. B. Studies with RP 56976 (Taxotere), a semisynthetic analogue of Taxol. *J. Natl. Cancer Inst.*, 83: 288–291, 1991.
- Bissery, M. C., Guenard, D., Gueritte-Voegelein, F., and Lavelle, F. Experimental antitumor activity of Taxotere (RP 56976, NSC 628503), a Taxol analogue. *Cancer Res.*, 51: 4845–4852, 1991.
- Bissery, M. C., Nohynek, G., Sanderink, G. J., and Lavelle, F. Docetaxel (Taxotere®): a review of preclinical and clinical experience. Part I. preclinical experience. *Anticancer Drugs*, 6: 339–368, 1995.
- Bruno, R., and Sanderink, G. J. Pharmacokinetics and metabolism of Taxotere™ (docetaxel). *Cancer Surv.*, 17: 305–313, 1993.
- Gaillard, C., Monsarrat, B., Vuilhorgne, M., Royer, I., Monegier, B., Sablé, S., Guénard, D., Gires, P., Archimbaud, Y., Wright, M., and Sanderink, G. J. Docetaxel (Taxotere®) metabolism in the rat *in vivo* and *in vitro*. *Proc. Am. Assoc. Cancer Res.*, 35: 428, 1994.
- De Valeriola, D., Brassine, C., Gaillard, C., Kettler, J. P., Tomiak, E., Van Vreckem, A., Frühling, J., Frydman, A., Kerger, J., Piccart, M., Chapelle, P., and Blanc, C. Study of excretion balance, metabolism and protein binding of ¹⁴C-radiolabelled Taxotere (TXT) (RP56976, NSC628503) in cancer patients. *Proc. Am. Assoc. Cancer Res.*, 34: 373, 1993.
- Vuilhorgne, M., Gaillard, C., Sanderink, G. J., Royer, I., Monsarrat, B., Dubois, and Wright, M. Metabolism of taxoid drugs. *ACS (Am. Chem. Soc.) Symp. Ser.*, 583: 98–110, 1995.
- Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R., and Waterman, M. R. The *P450* gene superfamily: recommended nomenclature. *DNA*, 6: 1–11, 1987.
- Guengerich, F. P. Roles of cytochrome P450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res.*, 48: 2946–2954, 1988.
- Zhou, X. J., Zhou-Pan, X. R., Gauthier, T., Placidi, M., Maurel, P., and Rahmani, R. Human liver microsomal cytochrome P450A isozymes mediated vindesine biotransformation. *Biochem. Pharmacol.*, 45: 853–861, 1993.
- Zhou-Pan, X. R., Zhou-Pan, X., Placidi, M., Maurel, P., Barra, Y., Rahmani, R. Involvement of human liver cytochrome P450 3A in vinblastine metabolism: drug interactions. *Cancer Res.*, 53: 5121–5126, 1993.
- Cresteil, T., Monsarrat, B., Alvinerie, P., Tréluyer, J. M., Vieira, I., and Wright, M. Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. *Cancer Res.*, 54: 386–393, 1994.
- Van der Hoeven, T. A., and Coon, M. J. Preparation and properties of partially purified cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase from rabbit liver microsomes. *J. Biol. Chem.*, 249: 6302–6310, 1974.
- Bradford, M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, 72: 248–254, 1976.
- Omura, T., and Sato, R. The carbon monoxide-binding pigment of liver microsomes. I Evidence of its hemoprotein nature. *J. Biol. Chem.*, 239: 2370–2378, 1964.

17. Nash, T. The coloric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, 55: 516-521, 1953.
18. Mieyal, J. J., and Blumer, J. L. Acceleration of the autooxidation of human oxyhemoglobin by aniline and its relation to hemoglobin-catalyzed aniline hydroxylation. *J. Biol. Chem.*, 251: 3442-3446, 1976.
19. Marre, F., Fabre, G., Lacarelle, B., Bourrie, M., Catalin, J., Berger, Y., Rahmani, R., and Cano, J. P. Involvement of the cytochrome P450IID subfamily in minaprine 4-hydroxylation by human hepatic microsomes. *Drug Metab. Dispos.*, 20: 316-321, 1992.
20. Dou, M., de Sousa, G., Lacarelle, B., Placidi, M., Lechene de la Porte, P., Domingo, M., Lafont, H., and Rahmani, R. Thawed human hepatocytes in primary culture. *Cryobiology*, 29: 454-469, 1992.
21. Commerçon, A., Bourzat, J. D., Bézard, D., and Vuilhorgne, M. Partial synthesis of major human metabolites of docetaxel. *Tetrahedron*, 50: 10289-10298, 1994.
22. Gires, P., Gaillard, C., Sanderink, G. J., Martin, S., Piguet, V., Kettler, J. P., Nichele, G., Martinet, M., Chapelle, P., and Bost, P. E. [¹⁴C]-Docetaxel (Taxotere[®]) disposition in the isolated perfused rat liver. *Eur. J. Drug Metab. Pharmacokinet.*, 19 (Suppl. 2): 29, 1994.
23. Babany, G., Larrey, D., and Pessayre, D. Macrolide antibiotics as inducers and inhibitors of cytochrome P-450 in experimental animals and man. In: G. G. Gibson (ed.), *Progress in Drug Metabolism*, Vol. 12, pp. 61-98. London: Taylor and Francis, 1988.
24. Guengerich, F. P., Martin, V. P., Beaune, P. H., Kremers, P., Wolff, T., and Waxman, D. J. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.*, 261: 5051-5060, 1986.
25. Back, D. J., and Tjia, J. F. Comparative effects of the antimycotic drugs ketoconazole, fluconazole, itraconazole and terbinafine on the metabolism of cyclosporin by human liver microsomes. *Br. J. Clin. Pharmacol.*, 32: 624-626, 1991.
26. Watkins, P. B. Noninvasive tests of CYP3A enzymes. *Pharmacogenetics*, 4: 171-187, 1994.
27. Kerlan, V., Dreano, Y., Bercovici, J. P., Beaune, P. H., Floch, H. H., and Berthou, F. Nature of cytochrome P450 involved in the 2-/4-hydroxylations of estradiol in human liver microsomes. *Biochem. Pharmacol.*, 44: 1745-1756, 1992.
28. Bargetzi, M. J., Aoyama, T., Gonzalez, F. J., and Meyer, U. A. Lidocaine metabolism in human liver microsomes by cytochrome P450III4. *Clin. Pharmacol. Ther.*, 46: 521-527, 1989.
29. Otton, S. V., Inaba, T., and Kalow, W. Competitive inhibition of sparteine oxidation in human liver by β -adrenoreceptor antagonists and other cardiovascular drugs. *Life Sci.*, 34: 73-80, 1984.
30. Guengerich, F. P., Müller-Enoch, D., and Blair, I. A. Oxidation of quinidine by human liver cytochrome P-450. *Mol. Pharmacol.*, 30: 287-295, 1986.
31. Cummings, J., Allan, L., Willmott, N., Riley, R., Workman, P., and Smyth, J. F. The enzymology of doxorubicin quinone reduction in tumour tissue. *Biochem. Pharmacol.*, 44: 2175-2183, 1992.
32. Bertillon, L., Henthorn, T. K., Sanz, E., Tybring, G., Säwe, J., and Villen, T. Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation type. *Clin. Pharmacol. Ther.*, 45: 348-355, 1989.
33. Andersson, T., Regardh, C. G., Dahl-Puustinen, M., and Bertillon, L. Slow omeprazole metabolizers are also poor S-mephenytoin hydroxylators. *Ther. Drug Monit.*, 12: 415-416, 1990.
34. Skjelbo, E., and Brosten, K. Inhibitors of imipramine metabolism by human liver microsomes. *Br. J. Clin. Pharmacol.*, 34: 256-261, 1992.
35. Lemoine, A., Gautier, J. C., Azoulay, D., Kiffel, L., Belloc, C., Guengerich, F. P., Maurel, P., Beaune, P., and Leroux, J. P. Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver. *Mol. Pharmacol.*, 43: 827-832, 1993.
36. Raucy, J. L., Lasker, J. M., Lieber, C. S., and Black, M. Acetaminophen activation by human liver microsomes P450IIE1 and P450IA2. *Arch. Biochem. Biophys.*, 271: 270-283, 1989.
37. Pichard, L., Fabre, I., Fabre, G., Domergue, J., Saint Aubert, J., Mourad, G., and Maurel, P. Cyclosporin drug interactions: screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab. Dispos.*, 18: 595-606, 1990.
38. Bruno, R., Vergniol, J. C., Montay, G., Le Bail, N., Frydman, A., Clavel, M., and Marty, M. Clinical pharmacology of Taxotere (RP56976) given as 1-2 hour infusion every 2-3 weeks. *Proc. Am. Assoc. Cancer Res.*, 33: 261, 1992.